Journal of Forensic and Legal Medicine 16 (2009) 64–66

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/jflm

Original Communication

Prevalence of dihydrocodeine in hydrocodone positive postmortem specimens

Amanda J. Jenkins PhD [a,*], Eric S. Lavins BS [b], Courtney Hunek BS [c,1]

[a] Lake County Crime Laboratory, 235 Fairgrounds Road, Painesville, OH 44077, USA
[b] The Office of the Cuyahoga County Coroner, Cleveland, OH, USA
[c] University of Toledo, Toledo, OH, USA

ARTICLE INFO

Article history:
Received 20 February 2008
Received in revised form 14 August 2008
Accepted 16 August 2008
Available online 26 September 2008

Keywords:
Forensic medicine
Toxicology
Hydrocodone
Dihydrocodeine

ABSTRACT

Hydrocodone (HC) has received renewed interest in the US due to reported increases in opiate related deaths involving psychotherapeutic drugs. The relative contribution of dihydrocodeine (DHC) in these deaths is unknown since little testing of this compound is performed. The objective of the study was to determine the prevalence of DHC in HC positive decedents and report the range of concentrations detected in these cases in order to evaluate the potential role of DHC in the deaths and determine the usefulness of including this analyte in opioid testing protocols. Specimens were assayed by liquid–liquid or solid phase extraction followed by gas chromatography/mass spectrometry operated in the selected ion monitoring mode. A multipoint calibration was utilized in the linear range 2–600 ng/mL. Accuracy for HC, DHC and hydromorphone (HM) was 101–106% and between day precision at 160 ng/mL between 7% and 11%.

One hundred and thirty six cases were identified with the majority male (62%) and white (83%). A search of HC positive cases identified 64 with DHC (47%). The range of HC concentrations was 9–3039 ng/mL heart blood ($n = 43$) and 42–12353 ng/mL urine ($n = 21$). DHC concentrations in these cases ranged 3–243 ng/mL in heart blood and 5–1842 ng/mL in urine. DHC/HC ratios ranged 0.00(7)–2.90 in blood ($n = 43$), and 0.01–5.04 in urine ($n = 21$) with 16% and 24% of these cases with ratios >0.50, respectively. HM was detected in only 9 HC cases with the majority positive in urine.

1. Introduction

The 2005 US National Survey on Drug Use and Health: National Findings[1] reported that 6.4 million individuals aged 12 or older had used prescription type psychotherapeutic drugs for non-medical purposes in the past month. Of these, more than 4 million people had used pain relievers. These individuals reportedly obtained the drugs "from a friend or relative for free" (59.8%).[1] The second most common source was a physician (16.8%). In recent years an increase in opiate related drug misuse deaths have been reported in many states.[2] Hydrocodone (HC), oxycodone and methadone have all been implicated in these deaths. The majority of full service postmortem forensic toxicology laboratories have the ability to screen, quantitate and confirm the presence of hydrocodone. Hydrocodone is metabolized by *O*- and *N*-demethylation and 6-keto reduction to produce 6-α and 6-β hydroxy metabolites.[3,4] These transformations produce hydromorphone (HM), norhydrocodone and hydrocodol. The latter exists as a stereoisomer, the 6-α isomer is also known as dihydrocodeine (DHC).[4] The unconjugated forms of these metabolites contribute to the pharmacological activity of the parent compound. Since HM is reportedly the most prevalent metabolite, especially in rapid metabolizers, and DHC is unavailable in the US, most toxicology laboratories do not include DHC in their opiate menus. The frequency of detection and possible contribution to HC related deaths is therefore, unknown.

The objective of the current study was to determine the prevalence of DHC in HC positive cases and report the range of concentrations detected in these cases in order to evaluate the potential contribution to opiate related deaths and determine the usefulness of including this analyte in opioid testing protocols.

2. Materials and methods

The Toxicology Laboratory database at The Office of the Cuyahoga County Coroner, Cleveland, OH, was searched for all HC positive postmortem cases in Cuyahoga County from June 2002 to February 2006. The concentrations of HC, DHC, and HM were recorded regardless of cause or manner of death. In addition, demographic characteristics including gender, race and age were collated.

Specimens were collected in sodium fluoride/potassium oxalate plastic conical tubes and stored refrigerated until analysis. The heart blood (through the anterior wall of the ascending aorta)

* Corresponding author. Tel.: +1 508 334 7296.
E-mail address: amanda.jenkins@umassmemorial.org (A.J. Jenkins).
[1] Present address: The Cleveland Clinic Foundation, Cleveland, OH, USA.

1752-928X/$ - see front matter © 2008 Elsevier Ltd and Faculty of Forensic and Legal Medicine. All rights reserved.
doi:10.1016/j.jflm.2008.08.002

and urine were collected at autopsy by needle and syringe with both organs in situ. Autopsies were performed by several pathologists within 24 h of receipt of the decedent. The samples were analyzed by liquid–liquid extraction using a previously published procedure[5] or by solid phase extraction. In both extraction protocols, nalorphine was used as the internal standard and multipoint calibration curves constructed for each analyte in the linear range 2–600 ng/mL. Coefficients of determination (r^2) were $\geqslant 0.99$ for each drug. A negative and positive control at 160 ng/mL were assayed with each batch. Following derivatization with *n*-methyl-*n*-TMS-trifluoroacetamide (MSTFA), the trimethylsilyl derivatives were subjected to gas chromatographic–mass spectrometric analysis with selected ion monitoring. All drugs were identified utilizing one quantifying and two qualifying ions. Accuracy at the control concentration was between 101% and 106% for each analyte. Between day precision, expressed as a percent coefficient of variation was 11%, 9%, and 7% for HC, HM, DHC, respectively ($n = 10$).

3. Results

This study identified 136 HC positive cases. The majority (62%) of decedents was male, and white (83%). Individuals ranged in age from 17 to 94 years with a mean age of 51.4 years and a median of 48.5 years. Approximately 75% of positive specimens were heart blood. In 72 cases, DHC was not detected in the HC positive case (53%). The range of HC concentrations in these cases was 10–1078 ng/mL heart blood ($n = 58$, mean ± SD, 82.9 ± 151.2, median = 37 ng/mL) and 13–422 ng/mL urine ($n = 14$, 76.7 ± 116.8, median 29.5 ng/mL). In the heart blood 13 cases had concentrations of HC >100 ng/mL, while in the urine 2 cases were >100 ng/mL.

DHC was detected in the remaining 64 HC positive cases. In these cases the range of HC concentrations was 9–3039 ng/mL heart blood ($n = 43$, 347.0 ± 447.5, 124 ng/mL) and 42–12353 ng/mL in urine ($n = 21$, 1982.4 ± 3467.6, 343 ng/mL). DHC concentrations in these cases ranged 3–243 ng/mL in heart blood (34.9 ± 42.1, 20 ng/mL) and 5–1842 ng/mL urine (242.1 ± 418.4, 89 ng/mL). In the heart blood 26 of the 43 cases had HC concentrations >100 ng/mL and 16 urine specimens were >100 ng/mL HC. DHC concentrations were >100 ng/mL in 2 blood specimens and 10 urine samples. Demographic characteristics between the two groups showed little variability. In both groups male gender and white race predominated. However, the mean and median age for the HC + DHC + group was lower than for the HC + DHC − group at 47.9, 44.5; 54.5, 50.5 years, respectively.

Six suicides in this population were related to HC intoxication. In all but 1 case DHC was also detected (83%). Heart blood concentrations of HC were 133–2234 ng/mL with corresponding DHC concentrations of 34–95 ng/mL. There were also 31 HC related accidental deaths. DHC was detected in 21 of these cases (68%). Blood concentrations of HC were 26–1663 ng/mL with corresponding DHC concentrations of 7–243 ng/mL. The majority of these cases were multiple drug intoxications (26/31).

DHC/HC ratios were calculated for the 64 positive cases. In heart blood, the DHC/HC ratios ranged from 0.00(7)–2.90, with a mean ± SD, of 0.36 ± 0.65 ($n = 43$) and a median of 0.16. The concentration of DHC in heart blood was greater than the corresponding HC concentration in 3 cases, resulting in ratios >2 (66/23; 87/30;19/9 ng/mL). In heart blood, 16% of cases had ratios >0.50 (7/43). In urine, the DHC/HC ratios ranged from 0.01 to 5.04, with a mean ± SD, of 0.54 ± 1.17 ($n = 21$) and a median of 0.20. The concentration of DHC in urine was greater than the corresponding HC concentration in 2 cases, resulting in ratios >2 (1842/365;322/124)). In urine, 24% of cases had ratios >0.50 (5/21).

A review of the HM results revealed 9 positive cases, a 6.6% positivity rate. HM was detected in the urine of 6 cases, the bile of 1 and the heart blood of 2 cases. In 1 case the blood HM concentration was 19 ng/mL with a corresponding HC level of 2562 ng/mL and DHC 155 ng/mL.

4. Discussion

This study has identified the presence of DHC in approximately 47% of HC positive cases. In comparison, HM was detected in only 6.6% cases. DHC was more likely to be found in blood compared with urine in contrast to HM. Higher HC concentrations were generally associated with the likelihood of detecting DHC. The mean heart blood HC concentration with DHC was 347 ng/nL compared with a mean of 83 ng/mL in DHC negative cases. This finding is reinforced with the observation that deaths due to HC intoxication were more likely to be positive for DHC. Reported postmortem blood concentrations of HC associated with intoxication were 130–7000 ng/mL.[4] Spiller[6] reported 17 HC intoxication deaths with a mean blood HC concentration of 520 ng/mL and a range of 120–1600 ng/mL. Concentrations of DHC in HC related deaths have not been reported. Concentrations of DHC associated with drug related death were of the order of 1000 ng/mL. In the current study, for the majority of cases the DHC concentration was lower than the corresponding HC concentration regardless of whether the matrix was blood or urine.

The potential for postmortem redistribution may affect the ability to detect drugs depending upon the site of collection. Postmortem redistribution (PMR) has not been widely reported for HC, HM or DHC. Findings for another opioid, morphine, have been inconsistent. Gerostamoulos and Drummer[7] failed to detect significant PMR of morphine and its metabolites in heroin-related deaths but other investigators have reported higher central blood morphine concentrations compared with peripheral samples.[8] Drugs with a volume of distribution (Vd) >3 L/kg are most likely to undergo PMR.[9] HC (3.3–4.7 L/kg) and HM (2.9 L/kg) may fall into this category but the Vd of DHC is reportedly 1.0–1.3 L/kg.[4] Therefore, if PMR does occur for HC and HM, lower DHC/HC ratios would be obtained by measuring heart compared with a peripheral blood sample. This would also result in a greater degree of detectability of these drugs since concentrations would be higher in the central sample. However, HM was detected in only 2 HC heart blood positive cases.

It appears that DHC is a more significant metabolite of HC in blood than HM. Only 9 cases were positive for HM in any matrix tested. The 6.6% positivity rate may be compared with that found in hair reported by Moore et al.[10] Four of 24 HC positive hair specimens contained HM (16.6%). The incidence of HM is dependent on the genetic polymorphisms of CYP2D6 influence on HC metabolism.[11] Approximately 7% of the white population are CYP2D6 poor metabolizers.[12] The phenotype status of the decedents in this study was unknown. Even though the majority of decedents were white, the relatively low percentage of this population that may have been poor metabolizers does not explain the low incidence of HM in HC positive cases. HC is also metabolized to norhydrocodone by CYP 3A4. This compound was not measured in the study. Hutchinson et al.[11] reported that about 40% of HC is eliminated by non-CYP pathways.

Generally, low levels of DHC were detected, and were lower than concentrations associated with DHC only deaths. Therefore, DHC probably plays little role in HC related deaths. However, concentrations of DHC in HC intoxication deaths appeared to differ with respect to the manner of death. DHC concentrations in suicides were lower than those observed in accidental deaths. This finding may reflect the acute nature of suicides compared with

accidental deaths in which bioaccumulation of drug and chronicity may have occurred. Therefore, measurement of DHC may have relevance in assisting in the determination of the manner of death.

In conclusion, this is the first report of blood and urine postmortem concentrations of DHC in HC positive cases. It appears that DHC is a more common metabolite of HC than HM. Determination of DHC may assist in the elucidation of the manner of death in medicolegal death investigations.

Conflict of interest statement

The authors do not have any financial interests or personal relationships which may bias the enclosed work.

References

1. Results from the 2005 National Survey on Drug Use and Health: National Findings. Rockville MD, Office of Applied Studies, Substance and Mental Health Services Administration, Department of Health and Human Services, September 2006. DHHS Publication No. SMA 06-4194.
2. Opiate-related drug misuse deaths in six states: 2003. In The New DAWN Report 19 2006. Rockville MD, Office of Applied Studies, Substance and Mental Health Services Administration, Department of Health and Human Services.
3. *Physicians' desk reference*. 61st ed. Montvale, NJ, Thomson PDR; 2007.
4. Baselt RC. *Disposition of toxic drugs and chemicals in man*. 6th ed. Foster City, CA: Biomedical Publications; 2002.
5. Jenkins AJ, Lavins ES. 6-Acetylmorphine detection in postmortem cerebrospinal fluid. *J Anal Toxicol* 1998;**22**:173–5.
6. Spiller HA. Postmortem oxycodone and hydrocodone blood concentrations. *J Forensic Sci* 2003;**48**(2):429–31.
7. Gerostamoulos J, Drummer OH. Postmortem redistribution of morphine and its metabolites. *J Forensic Sci* 2000;**45**(4):843–5.
8. Bogusz M. Postmortem distribution pattern of morphine and morphine glucuronides in heroin overdose. *Int J Leg Med* 1997;**110**(2):114–6.
9. Yarema MC, Becker CE. Key concepts in postmortem drug redistribution. *Clin Toxicol* 2005;**43**:235–41.
10. Moore C, Feldman M, Harrison E, Sumandeep R, Coulter C, Kuntz D, et al. Disposition of hydrocodone in hair. *J Anal Toxicol* 2006;**30**:353–9.
11. Hutchinson MR, Menelaou A, Foster DJ, Coller JK, Somogyi AA. CYP2D6 and CYP3A4 involvement in the primary oxidative metabolism of hydrocodone by human liver microsomes. *Br J Clin Pharmacol* 2004;**57**(3):287–97.
12. Schmidt H, Vormfelde S, Klinder K, Gundert-Remy U, Gleiter CH, Skopp G, et al. Affinities of dihydrocodeine and its metabolites to opioids receptors. *Pharmacol Toxicol* 2002;**91**(2):57–63.